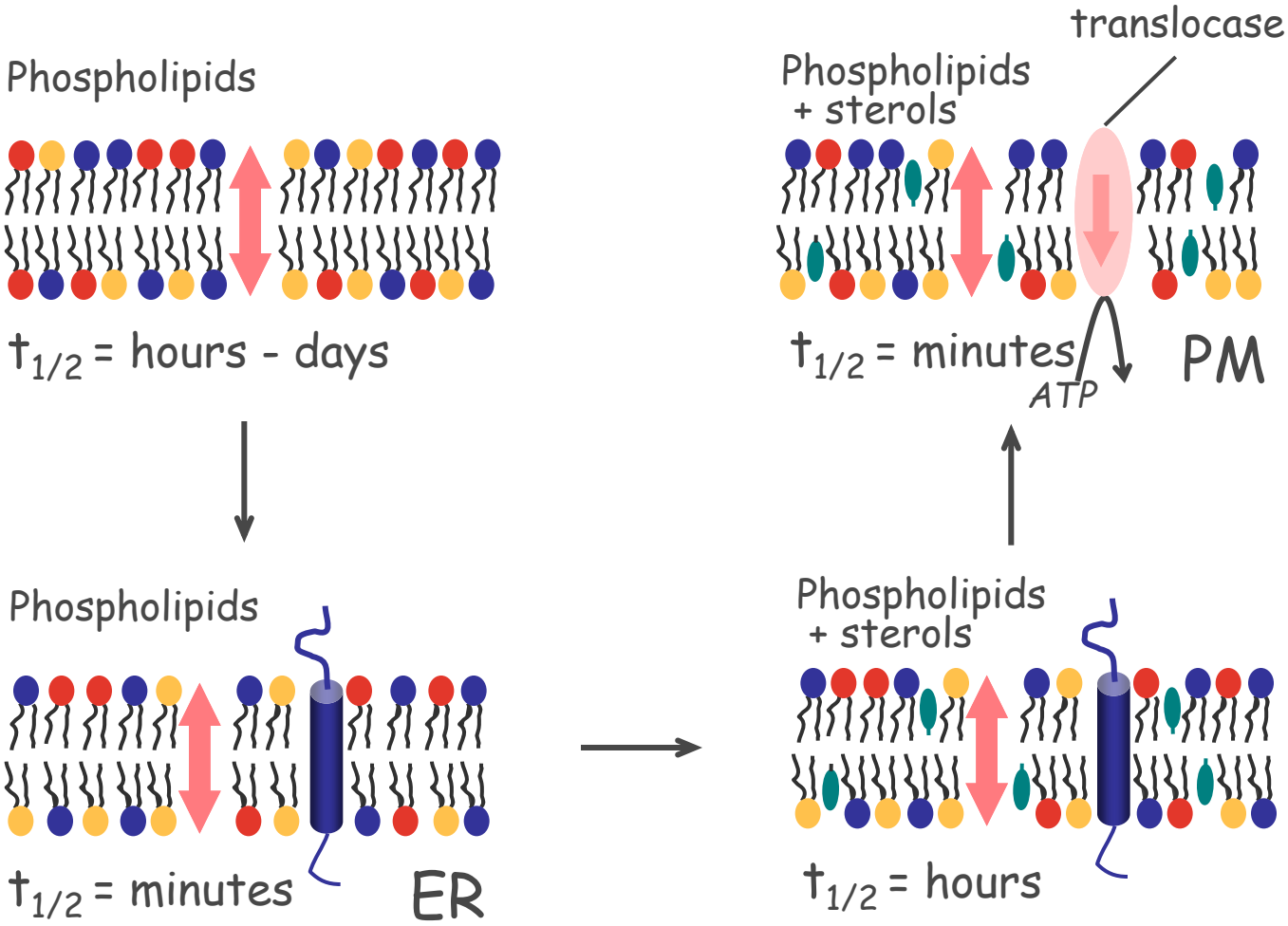
Lipid motions in membranes

Transbilayer flip-flop and phospholipid asymmetry

Interbilayer exchange and lipid transfer proteins

Membrane proteins accelerate flip-flop



Phospholipid asymmetry

- Sites of lipid synthesis are highly asymmetric

PC	Cytosolic side of endoplasmic reticulum
PE	
PS	
PI	
Glucosylceramide	Cytosolic side of Golgi
Sphingolipids	Luminal side of ER or Golgi

⇒Newly made lipids have to redistributed over both leaflets to:

- Preserve membrane integrity
- Create final lipid asymmetry in other membranes

Phospholipid asymmetry of plasma membrane

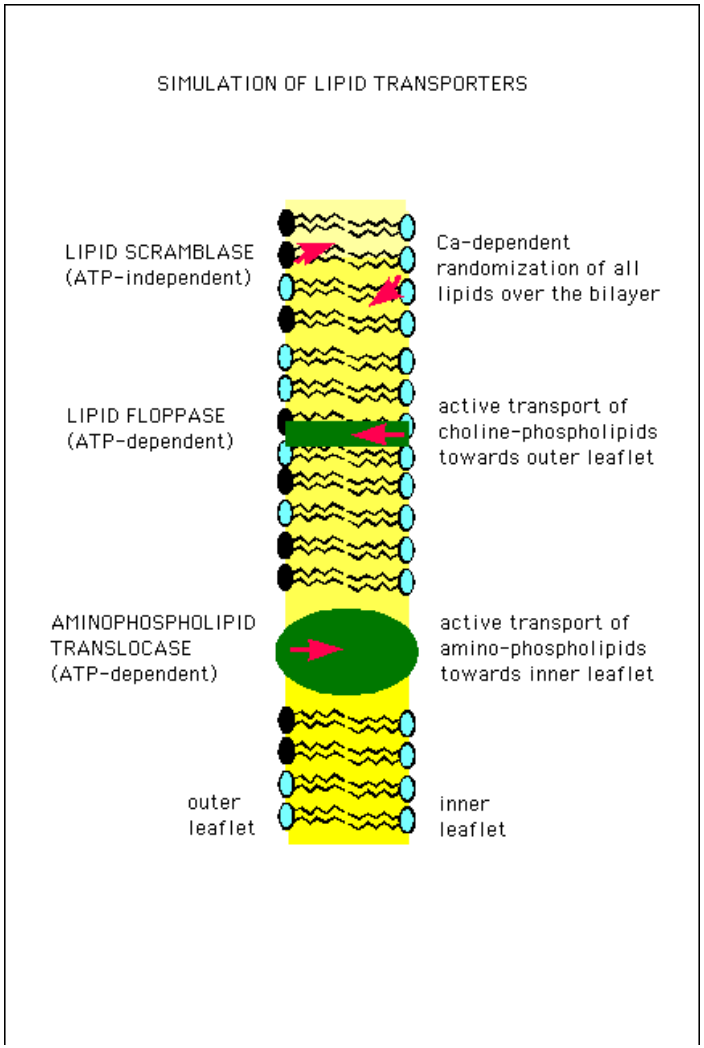
Human erythrocyte membrane phospholipid asymmetry:

Outside

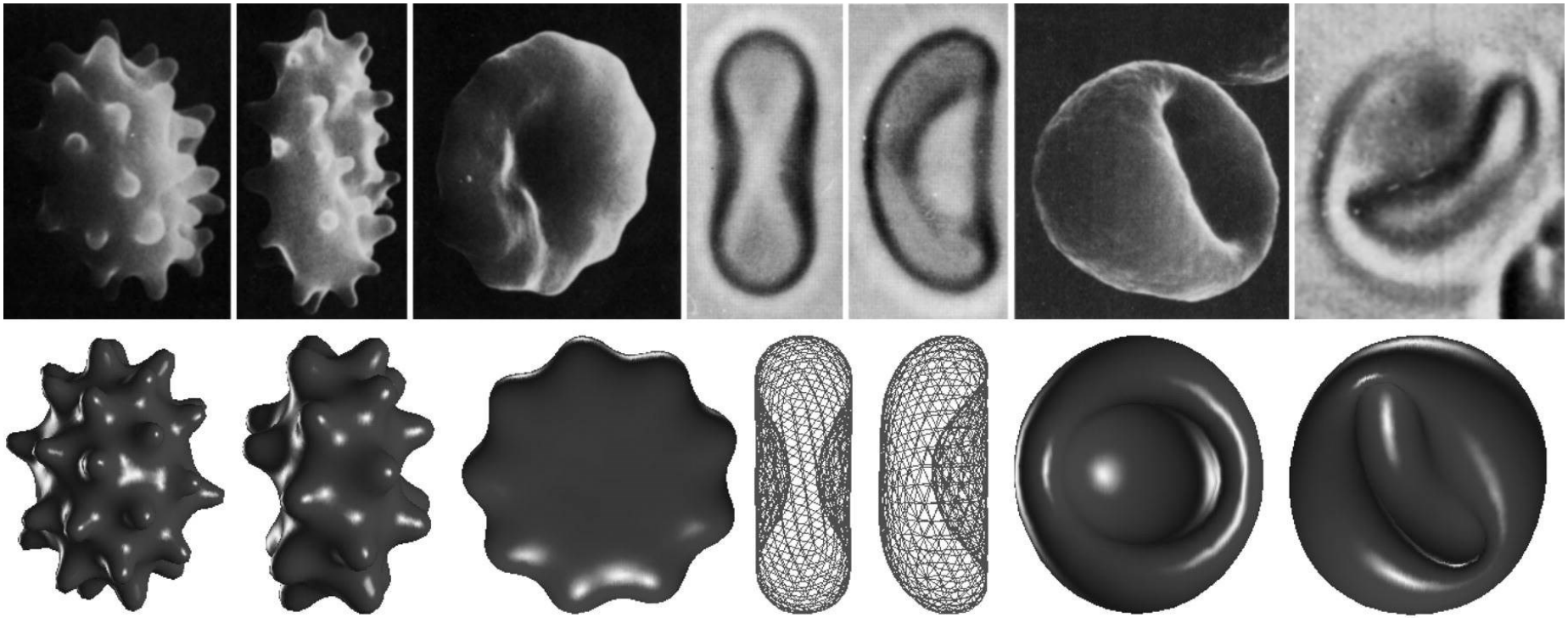
Inside

=> aminophospholipids inside 100 % for PS

=> "choline lipids" outside



Lipid asymmetry and Erythrocyte shape



Excess in outer leaflet

echinocyte

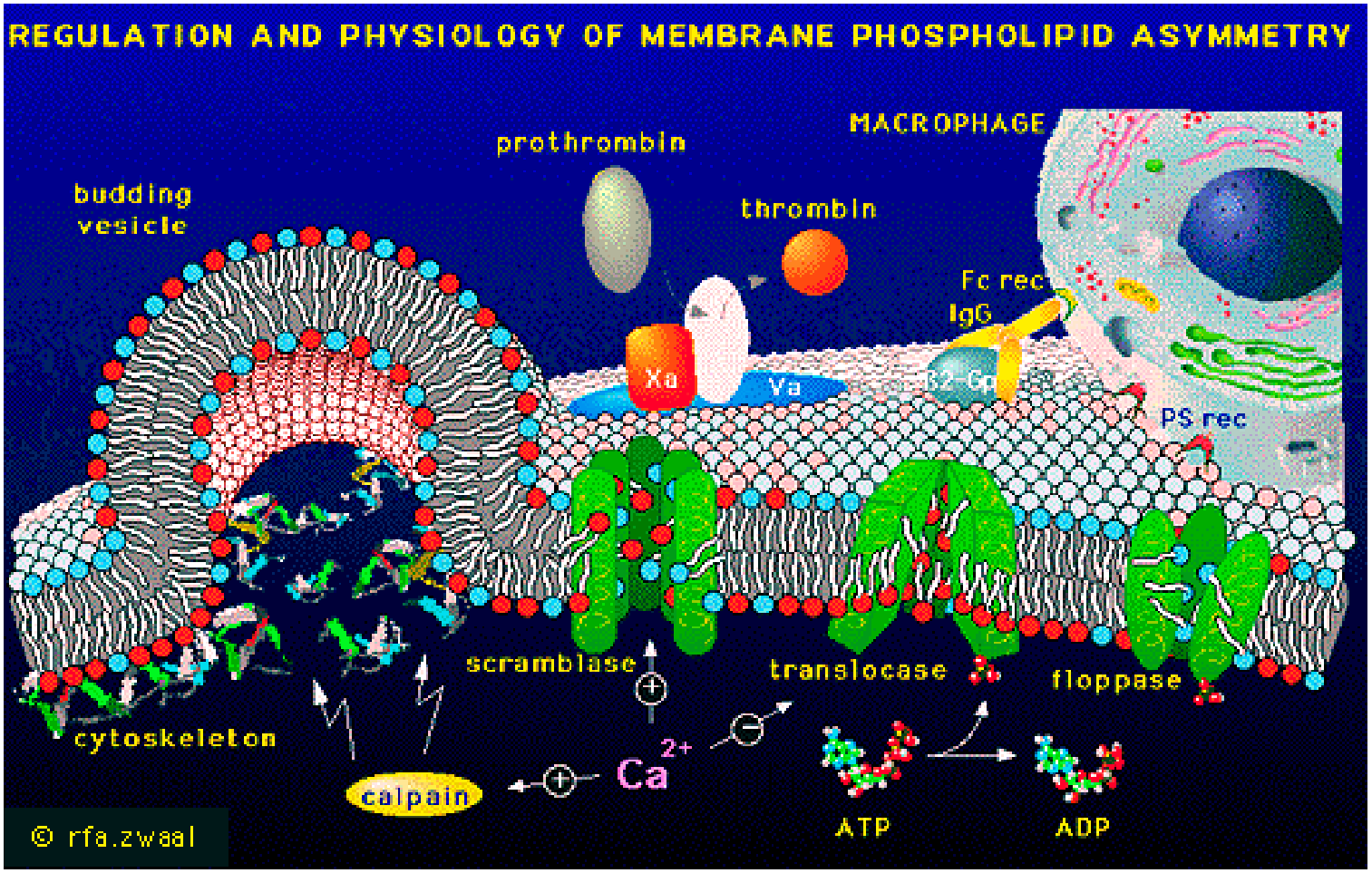
Normal balance

Excess in inner leaflet

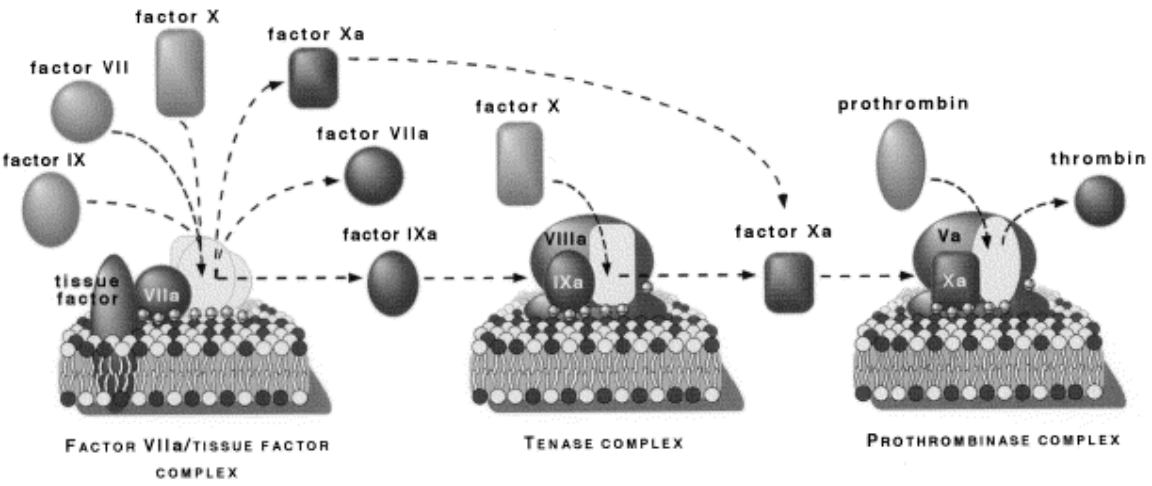
discocyte

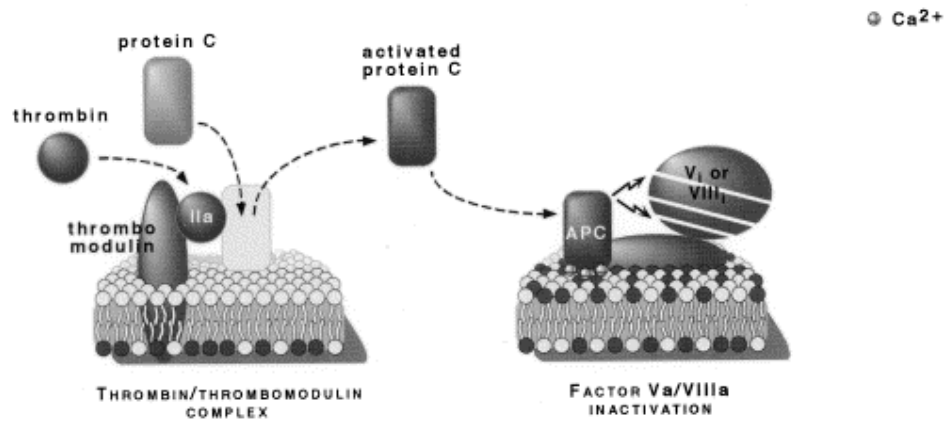
=> Balance between bilayer leaflets of total lipid and of lipid species

Physiological effects of PS exposure

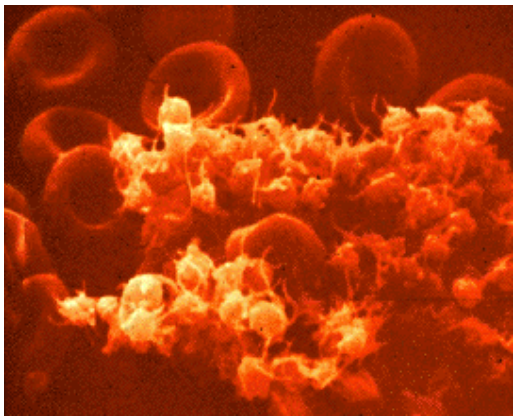


PS exposure and blood coagulation

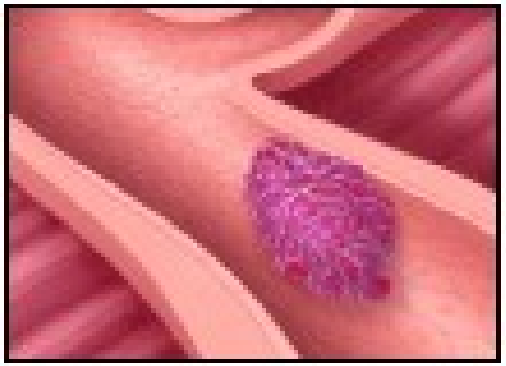




=> Platelet aggregation

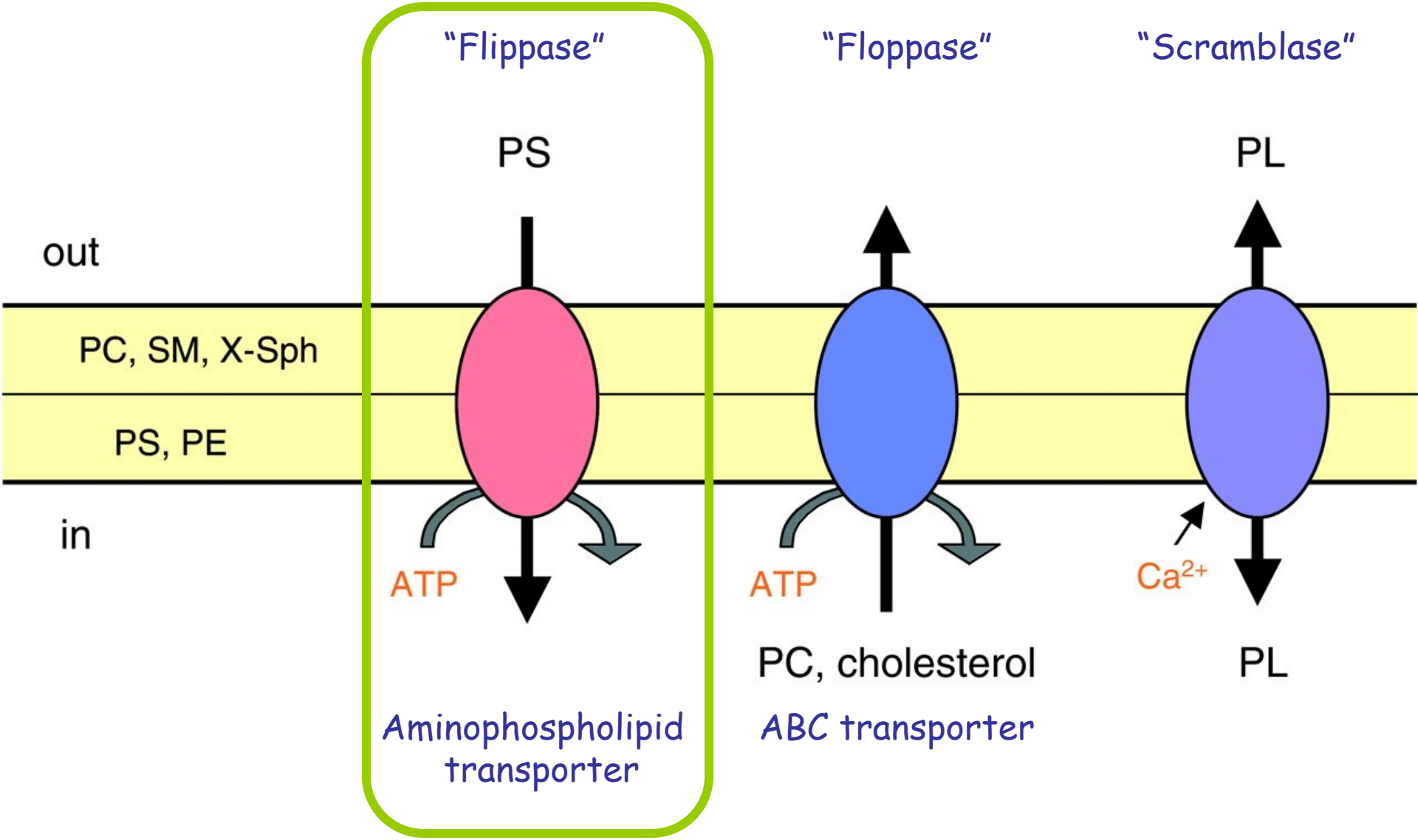


=> Thrombosis

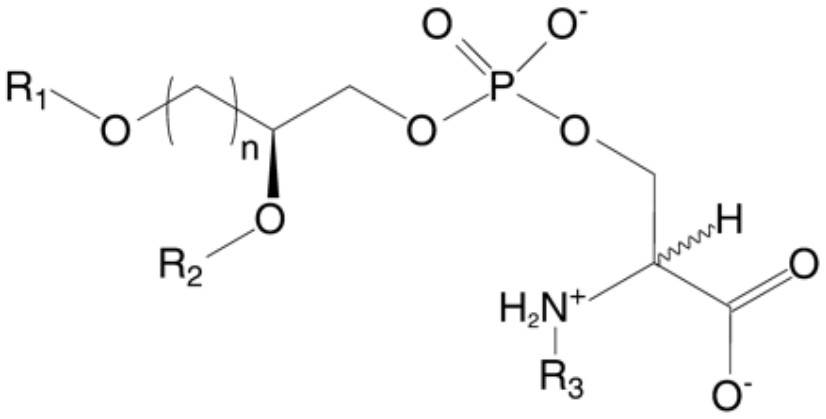


CLOTTING MECHANISM

Maintenance of plasma membrane phospholipid asymmetry

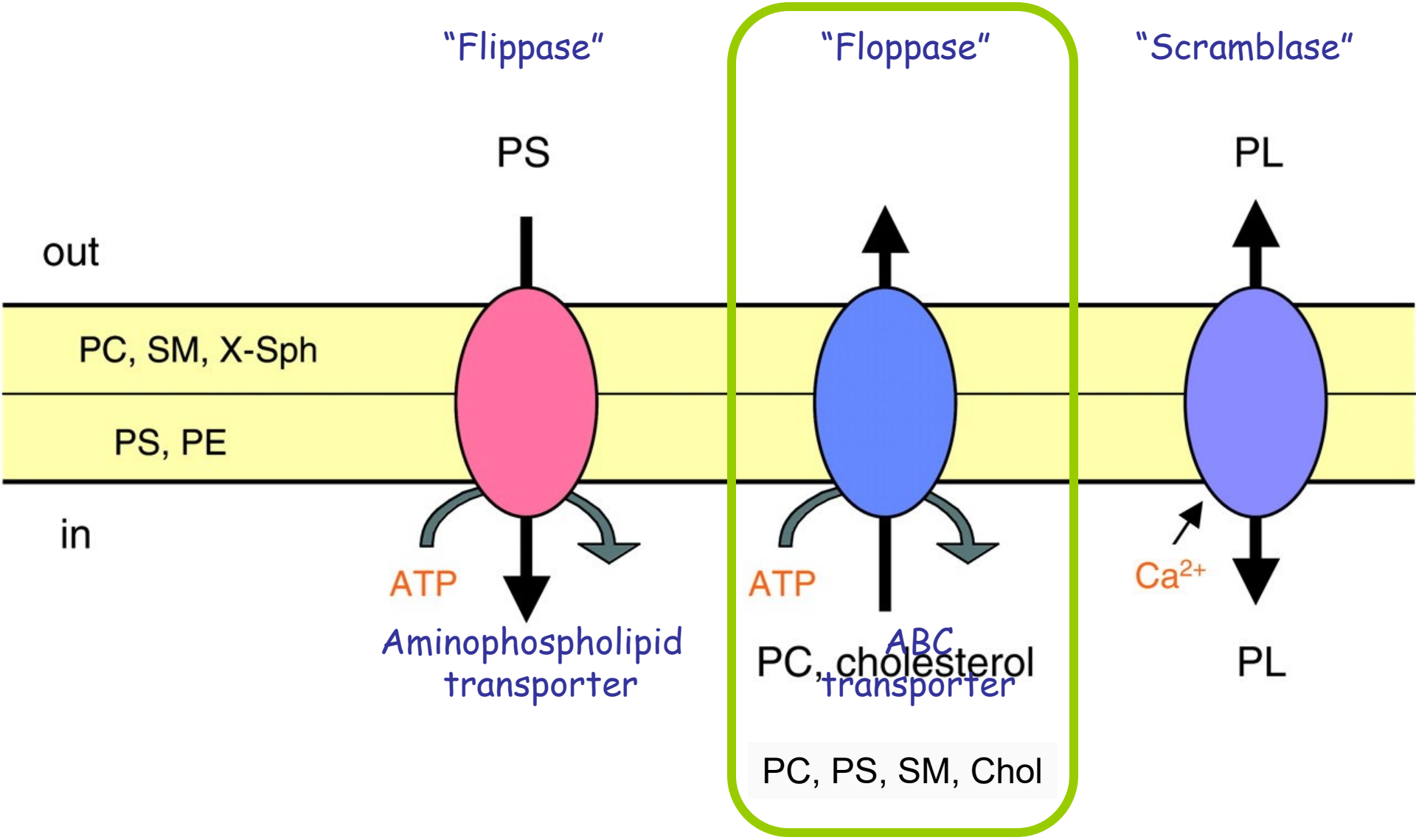


Mg^{2+}ATPase flippase substrate specificity



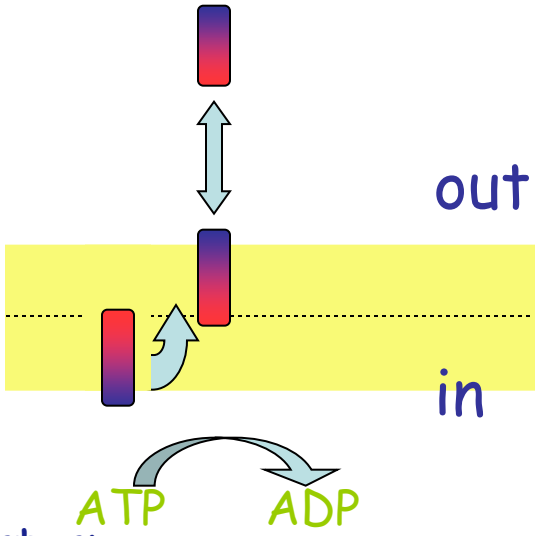
- PS is preferred over PE by a factor of 10
- The amine group is essential
- D- and **L-serine** as head group yield equal substrates
- sn-2,3 lipids are no substrates
- Butanetriol and glycerol as backbone yield equal substrates

Maintenance of phospholipid asymmetry



Floppases: ABC-transporters

- ATP-dependent exofacial or outward flux of lipids



- ABC-transporter:
  - Transmembrane protein performing active transport
  - ATP-hydrolysis provides energy **and** is coupled to transport
  - **A**TP-**B**inding **C**assette

Floppases: ABC-transporters

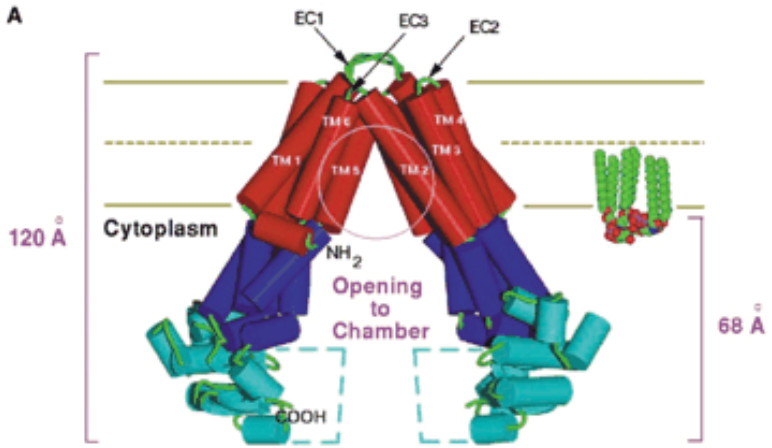
- Multi-drug resistance protein MsbA
  selective transport of Lipid A towards outer membrane

Side view

Transmembrane domain

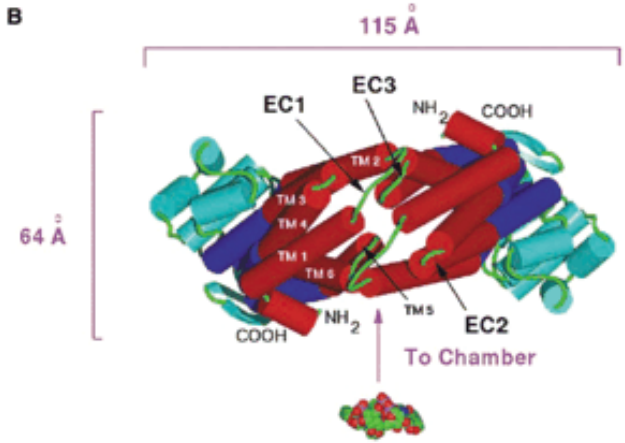
Intracellular domain

ABC



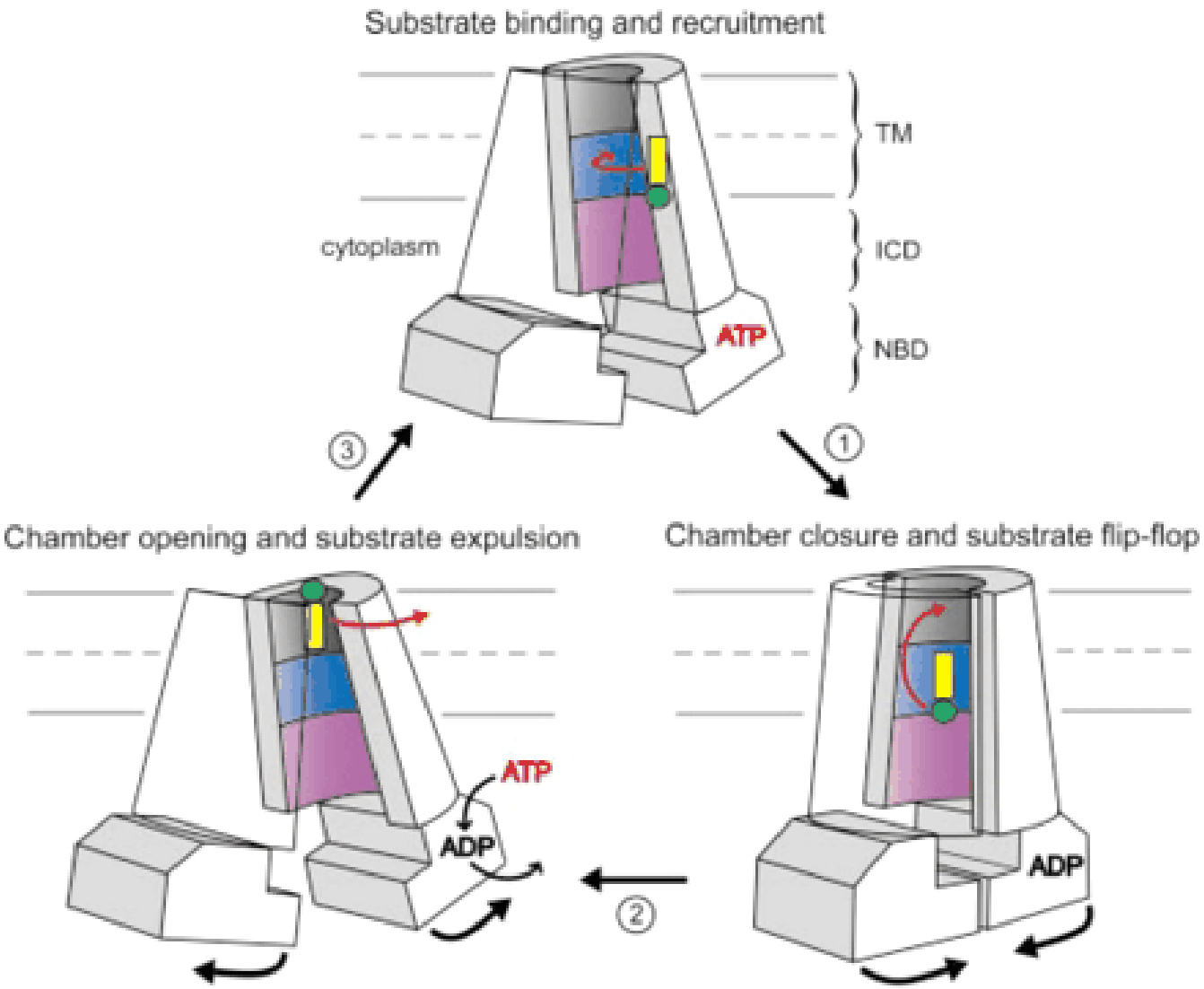
Lipid A

Top view



Floppases: ABC-transporters

- Proposed mechanism of transport cycle of MsbA

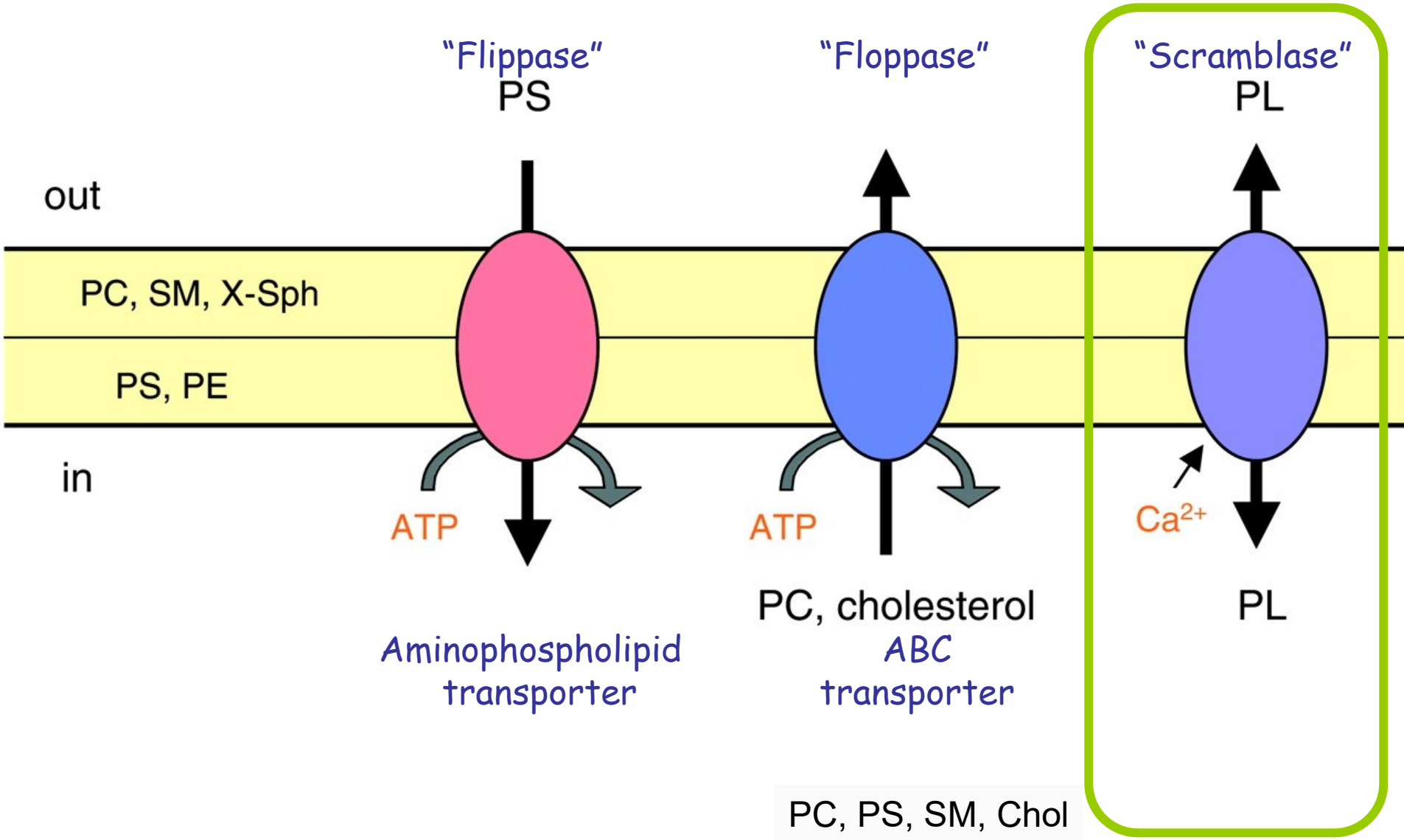


ABC-transporters as putative lipid floppases

Protein	Substrate specificity:	Role in:
MsbA	Lipid A	Asymmetry, export
ABC_A1	PS Cholesterol	Asymmetry Export
ABC_B1	Short chain PC, PE, Sph Labelled PS	Detoxification?
ABC_B4	PC	Asymmetry or export ?
ABC_C1	PC, SM Short labelled lipids	Asymmetry Detoxification?

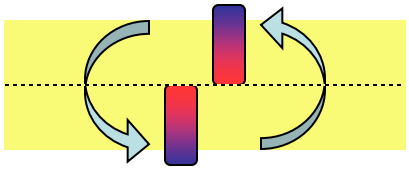
=> Involvement in flop, but often aimed to export.

Maintenance of phospholipid asymmetry



Scramblases

- Decrease of transbilayer asymmetry



÷ Endoplasmic reticulum

Constitutive non-selective flip-flop activity

=> redistribution of newly synthesized lipid

÷ Plasma membrane

Ca^{2+}-activated scrambling to present PS on cellular surface

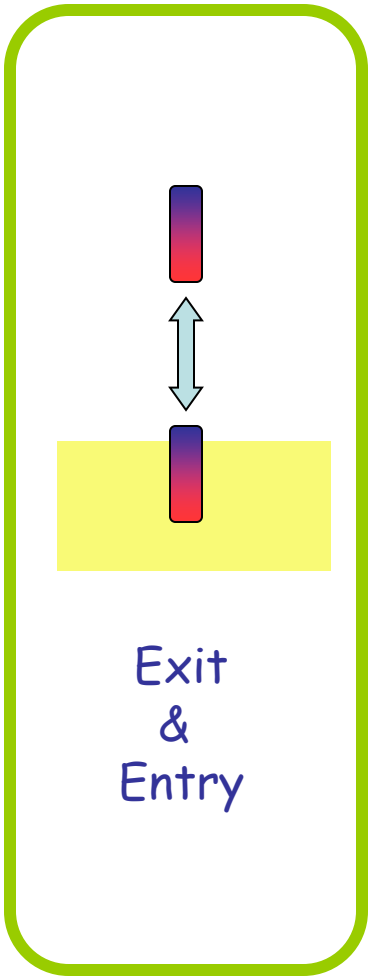
=> blood coagulation

=> apoptosis and macrophage engulfment

Exit and entry of lipids

- Transfer of a lipid molecule from membrane to water is highly unfavorable:
  - ÷ $\Delta G \approx +30$ kcal/mole
  - ÷ cmc ≈ 10-11^{-11} to 10^{-12} M

⇒Intermembrane lipid transfer needs
  - ÷ a catalyst!
  - ÷ closely apposed membranes



Phospholipid transfer proteins

- Phase-transfer catalyst
- Modulators of lipid metabolism

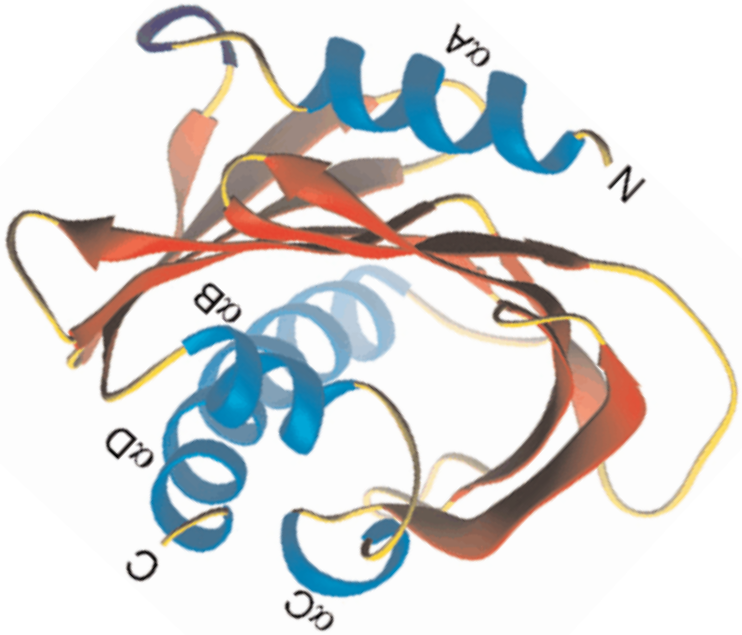
» PC-TP PC-specific transfer protein

» PI-TP PI transfer protein

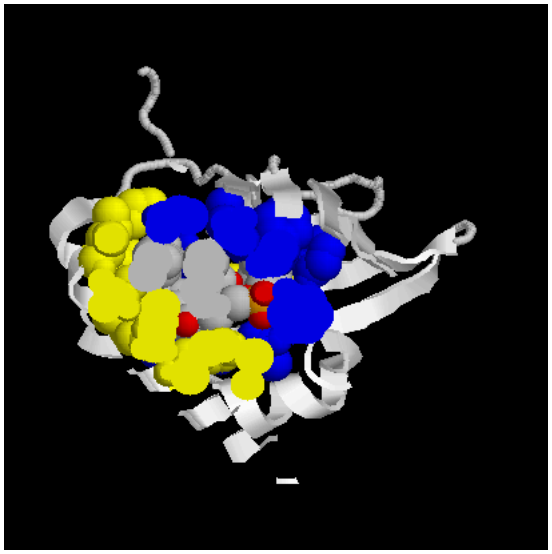
» nsLTP non-specific lipid TP or sterol carrier protein-2

scp2

Structure and mechanism of PC-TP



- PC molecule enveloped by PC-TP.
- PC's acyl chains surrounded by hydrophobic amino acids.
- PC's phosphate group surrounded by polar amino acids.



Structure and mechanism of PC-TP

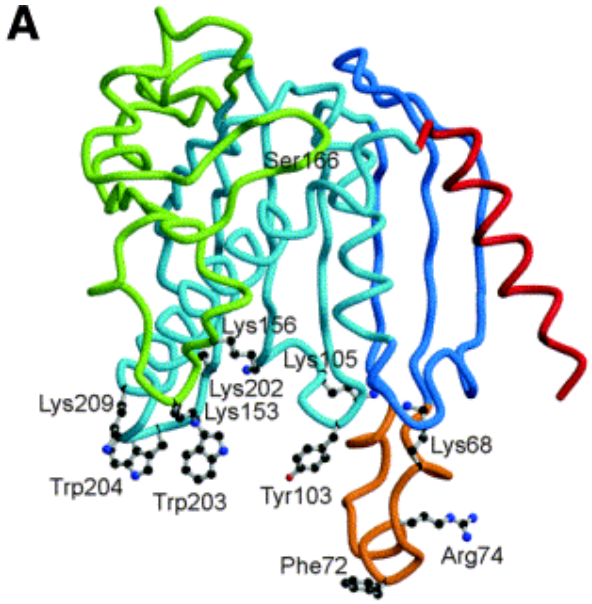
Coordination:

- Phosphate by Arg, Asp and Gln
- Me_3N^+ by aromatic cage

Structure of lipid within PC-TP

- grid: solvent accessible space
- structures rotated by 90°

Structure and mechanism of PI-TP

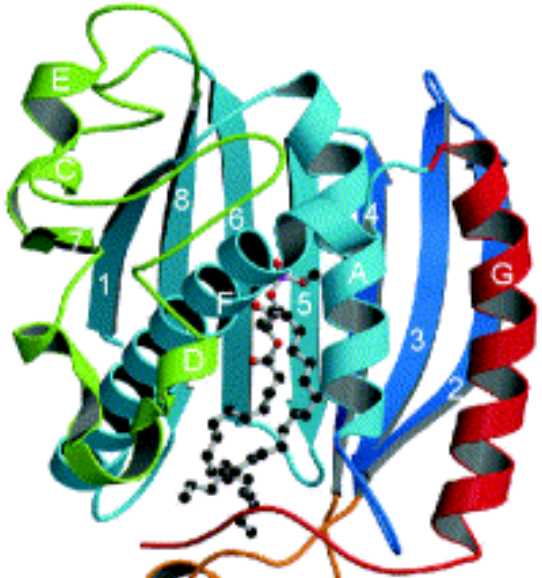


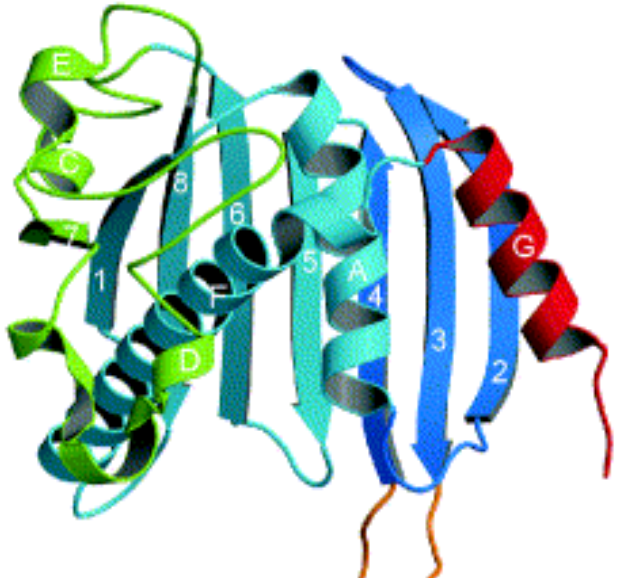
Ser166: Regulation by protein kinase C, phosphorylation inactivates

Cavity: polar top & apolar cylinder

Lys & Arg: Membrane binding

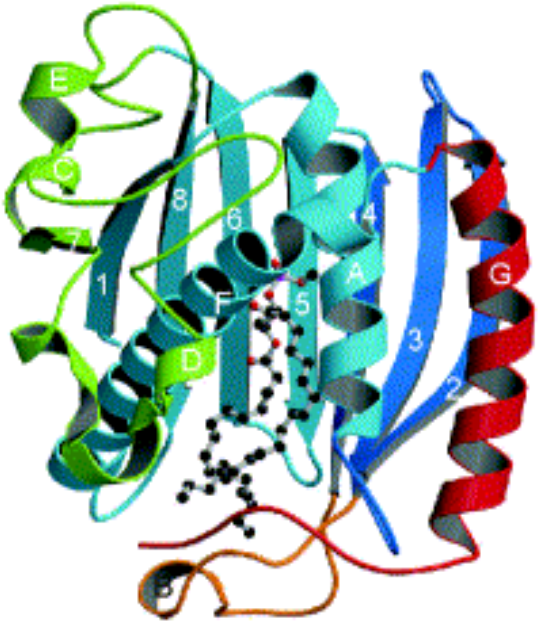
$W^{203,204}$: Membrane insertion



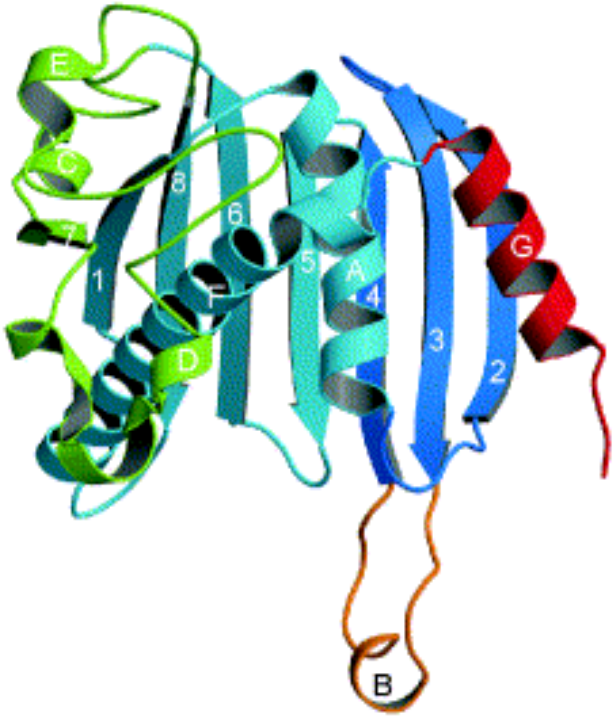


Structure and mechanism of PI-TP

Closed containing lipid

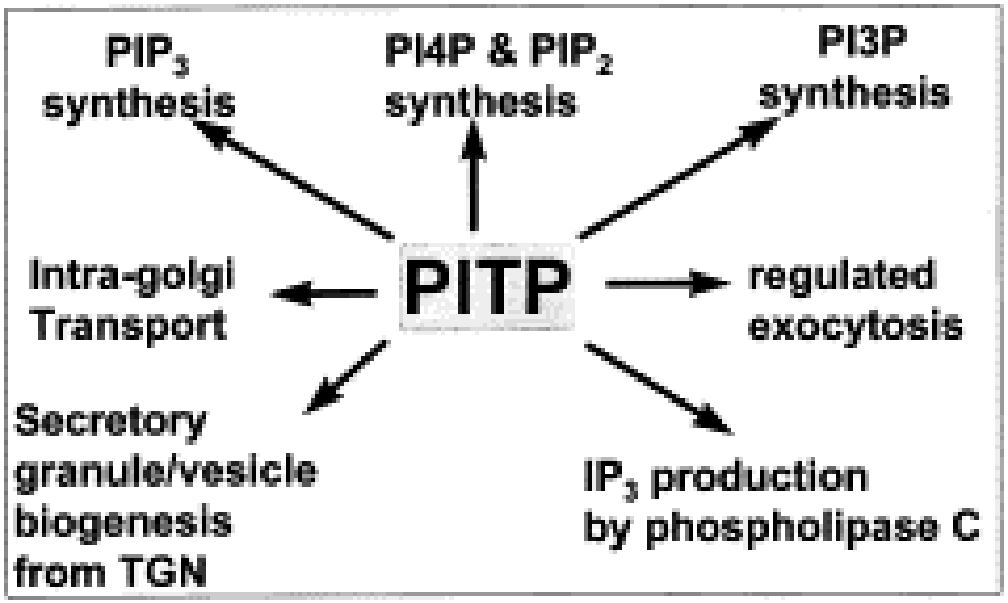


Open

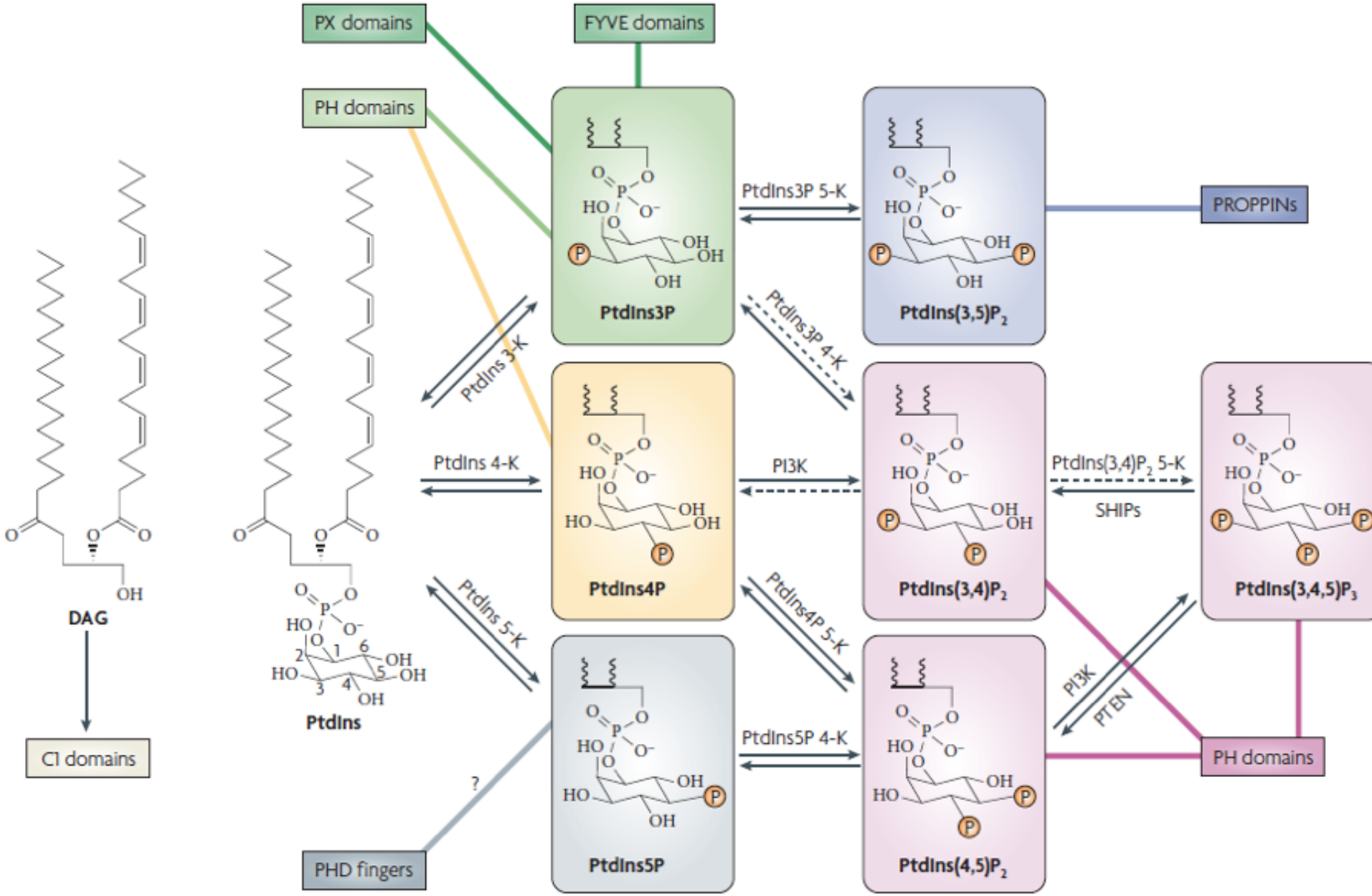


PI and PI-TP are central in cellular processes

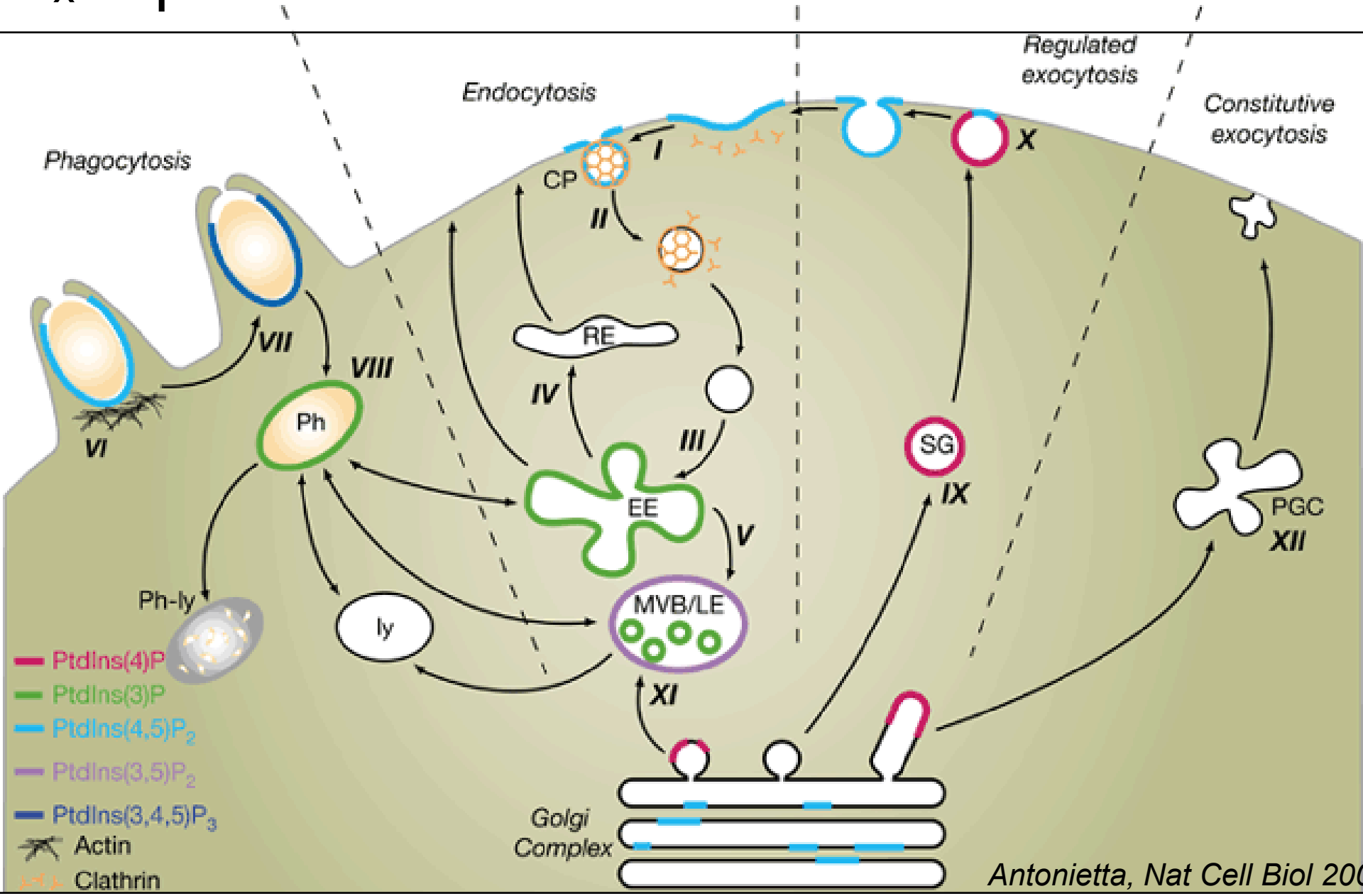
- **MULTIPLE FUNCTIONS OF PITP**



PIP lipids are central in cellular processes

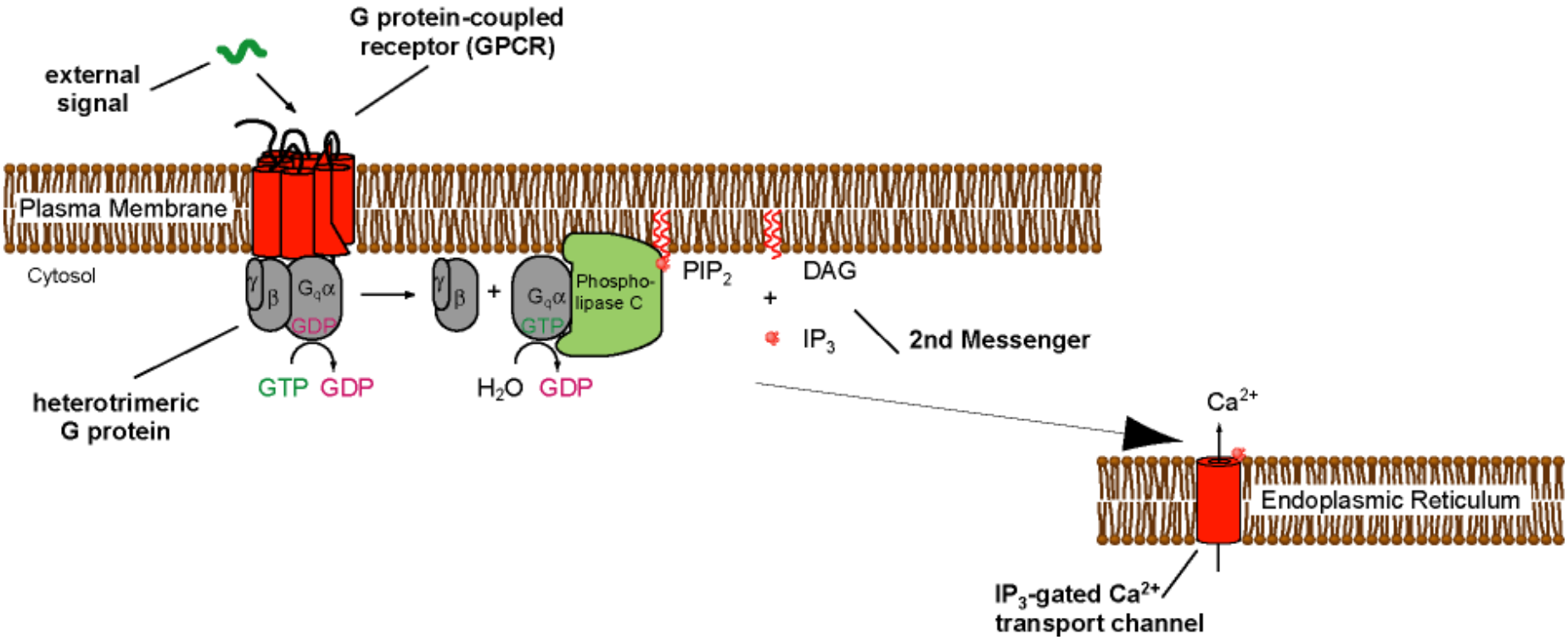


IP_x: Specific locations



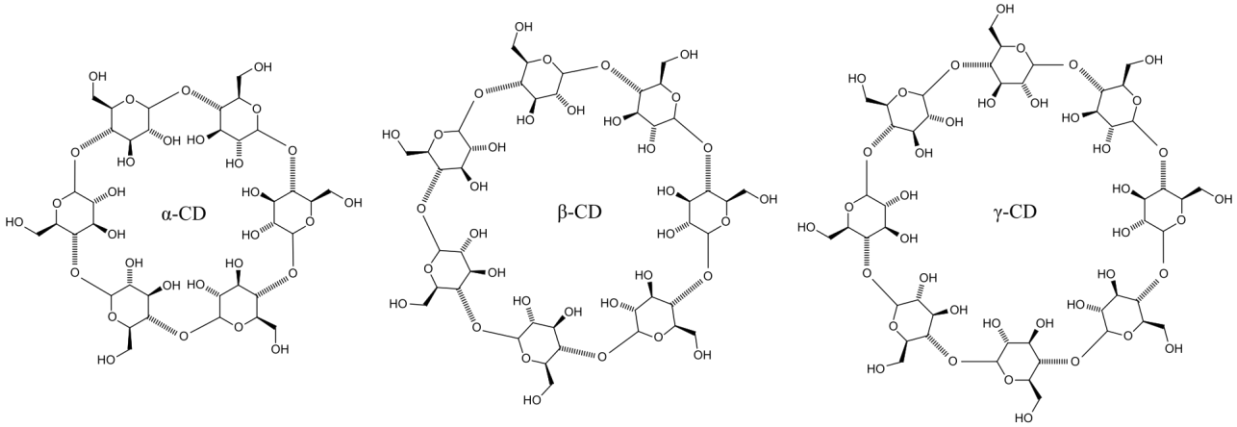
PI and PI-TP are central in cellular processes

- *G protein-coupled receptor signalling*



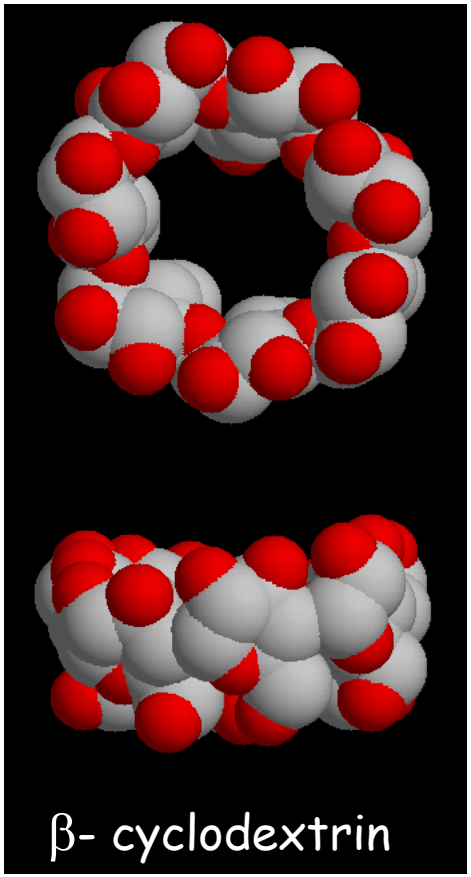
Artificial lipid carriers: Cyclodextrins

- Water-soluble shuttles of lipophilic compounds



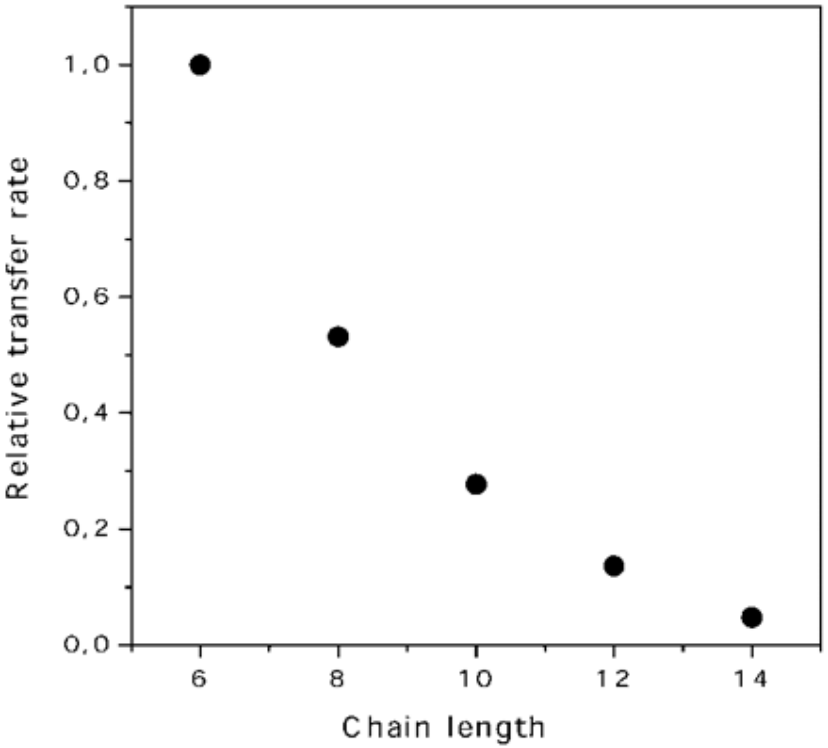
γ-cyclodextrin
8-mer

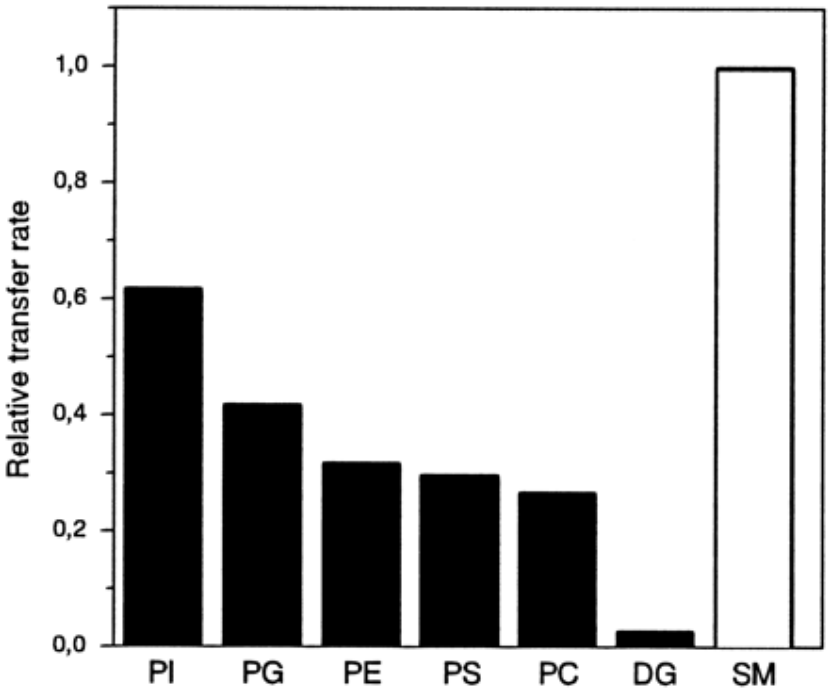
β- cyclodextrin
7-mer



Intervesicle lipid exchange catalyzed γ-CD

- Dependence of exchange rate on:
  - ÷ chain length
  - ÷ polar head group

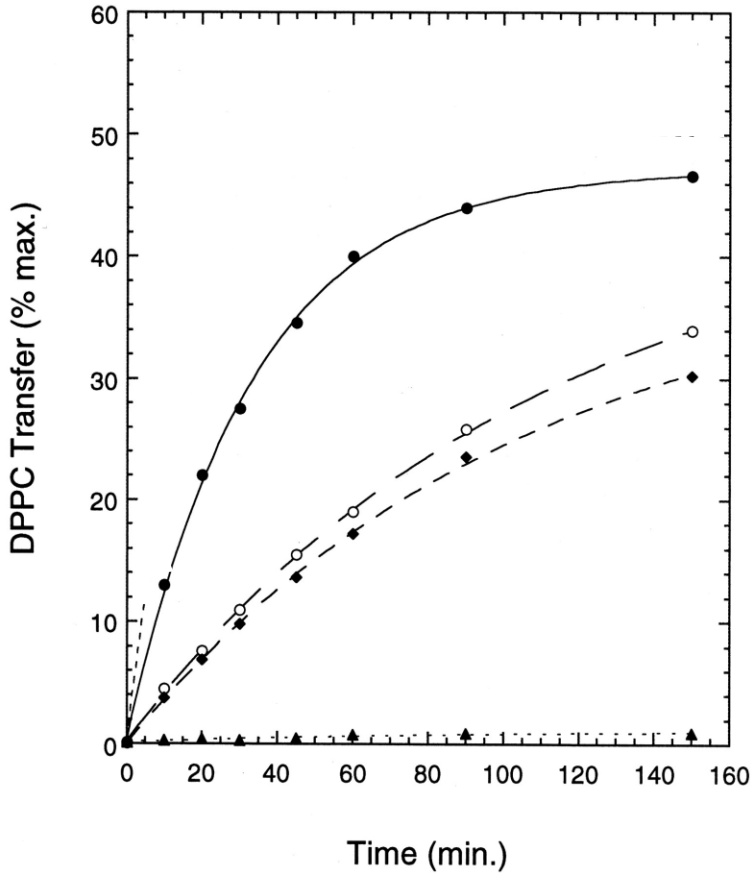




=> The more hydrophobic, the slower.

Intervesicle lipid exchange catalyzed CD

- Dependence of exchange rate on:
  ÷ cyclodextrin size.



α-CD 6-mer

β-CD 7-mer
γ-CD 8-mer

none

Phospholipids on the move II

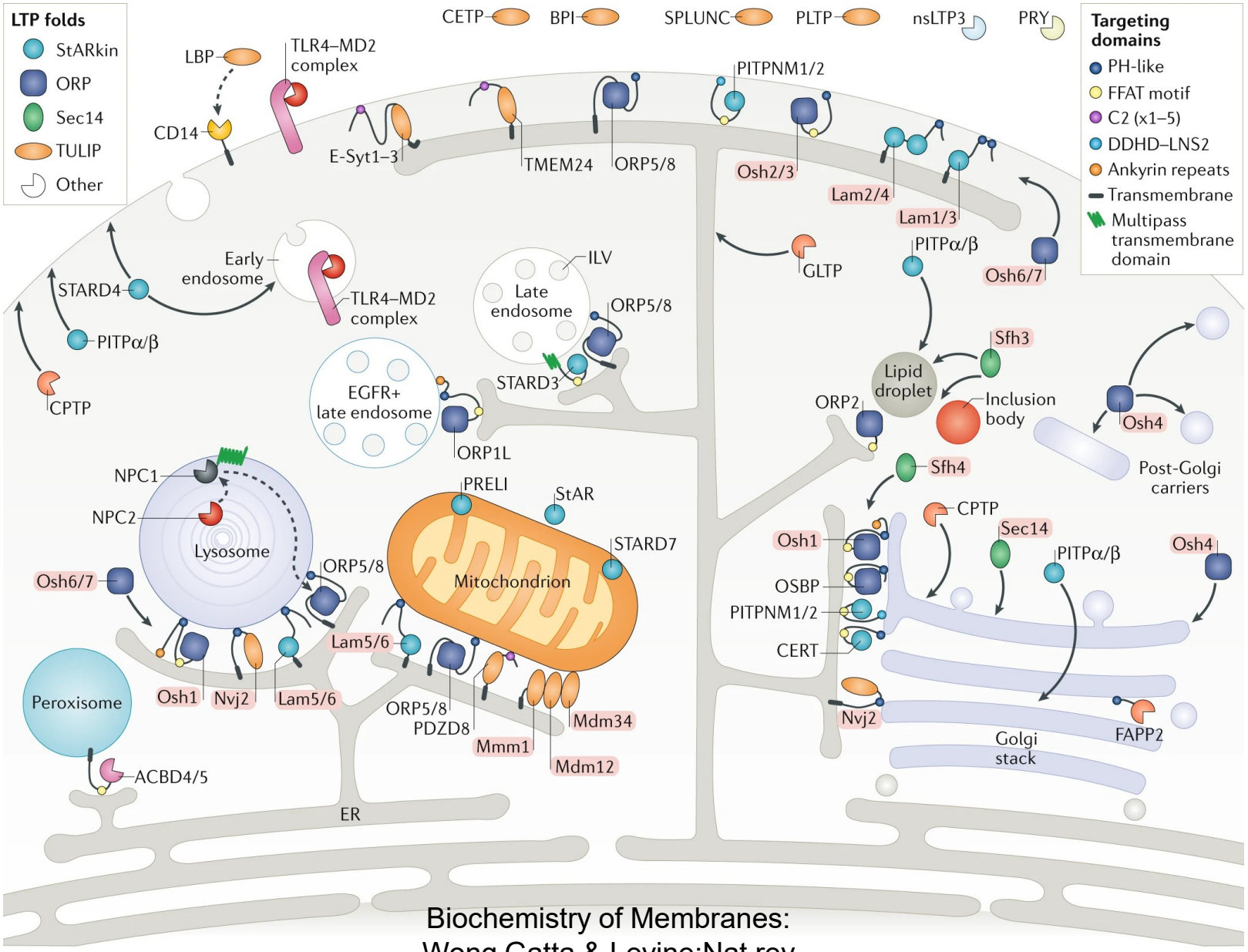
Text books:

Biomembranes – Gennis parts ofchapters Chpt 4, 5.1-4

Stryer parts ofchapters 12 & 26

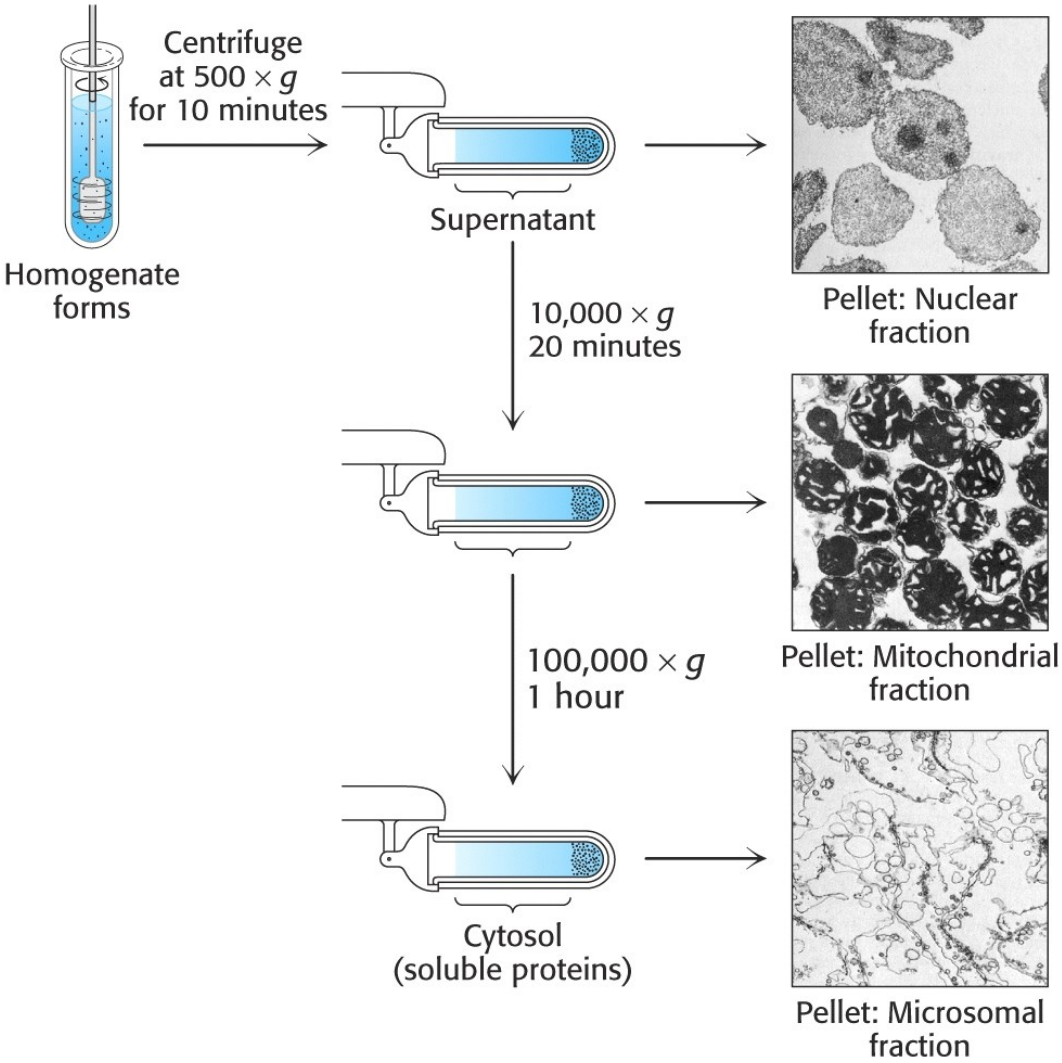
Review articles:

Wong Gatta & Levine;Nat rev MolCell Biol Feb 2019

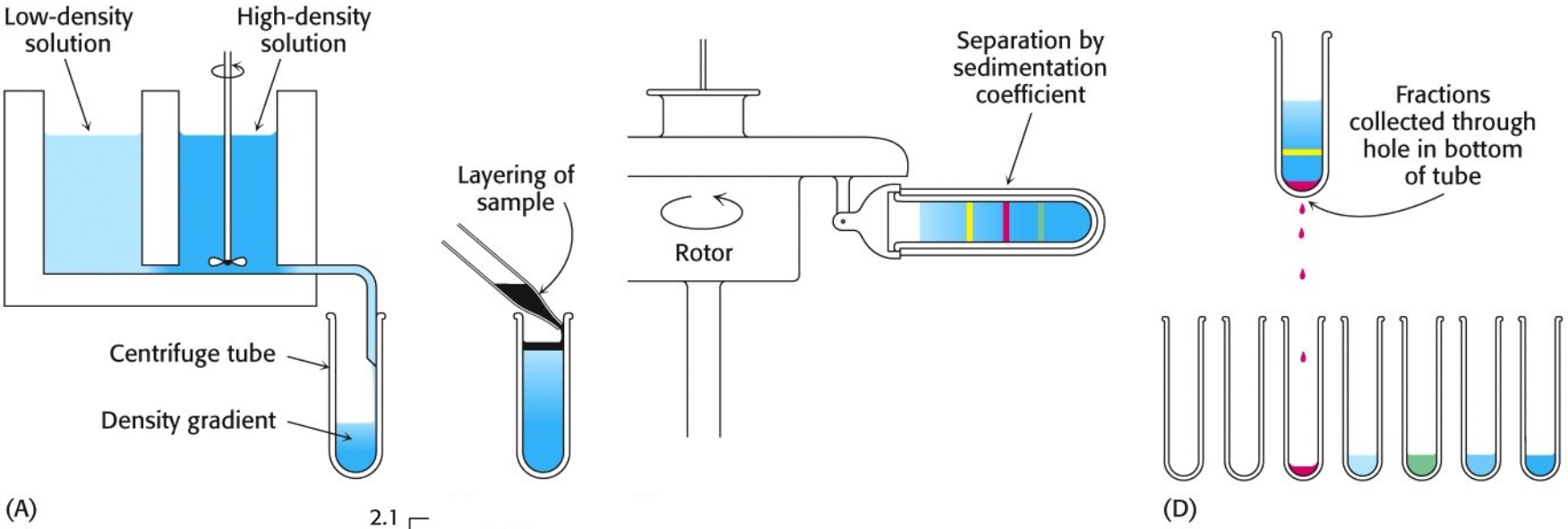


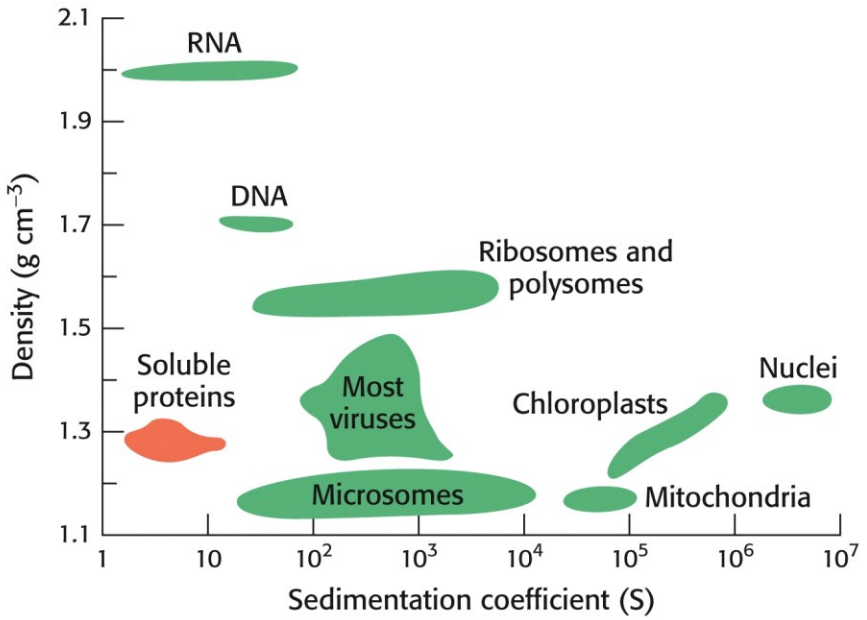
Biochemistry of Membranes:
Wong Gatta & Levine;Nat rev
MolCell Biol Feb 2019

Cell fractionation by differential centrifugation

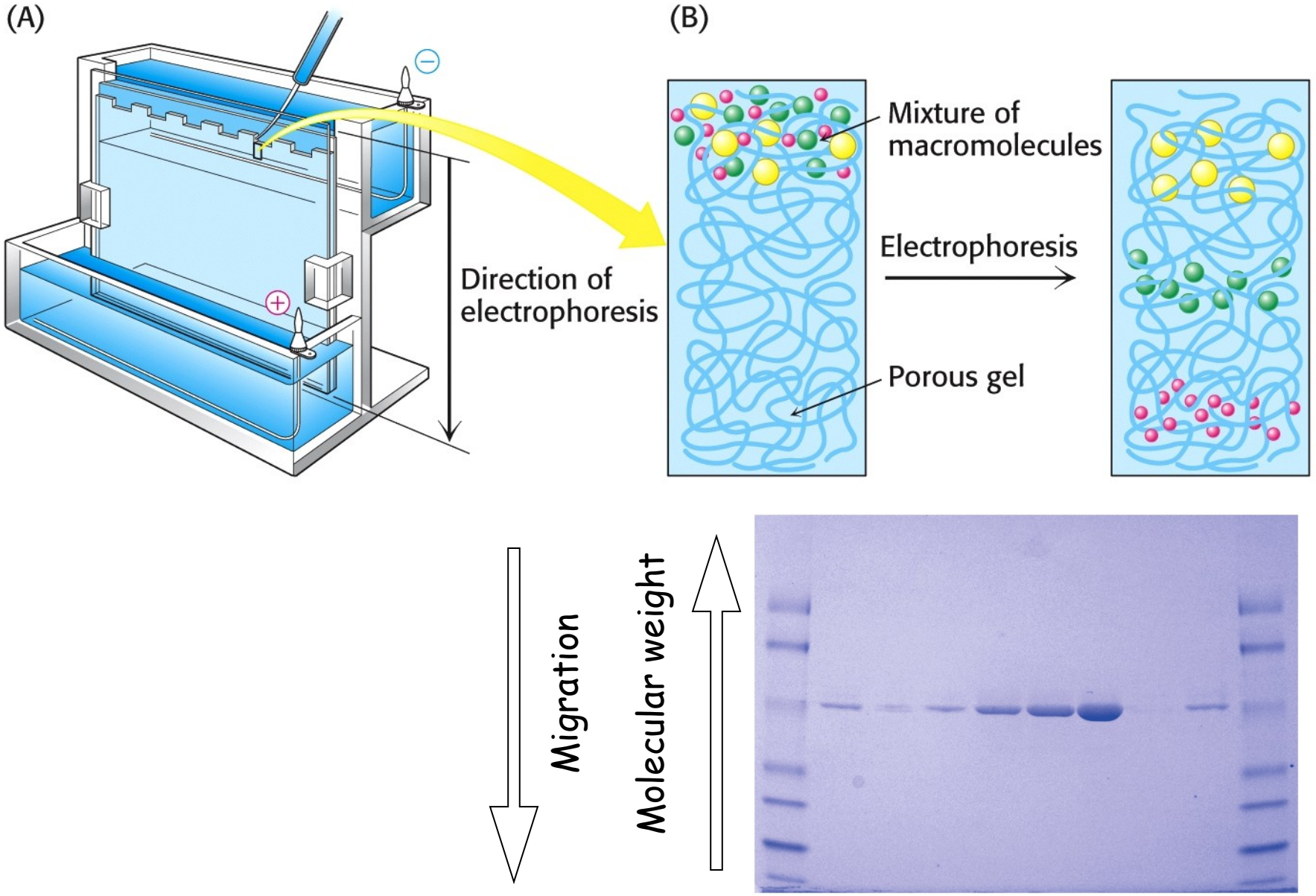


Cell fractionation by density gradient centrifugation





Protein separation by gel electrophoresis



In vitro transcription-translation-translocation assay

Components needed:

- DNA encoding protein
- Transcription machinery
- Translation machinery
- Membranes
- Energy source
- Cytosolic components